Monoclonal Antibodies—Therapeutic and Diagnostic Uses in Malignancy

JAMES N. LOWDER, MD, *and* RONALD LEVY, MD, *Stanford, California*

Murine monoclonal antibodies represent an attractive type of antitumor therapy because of their potential for exquisite specificity, production in large, pure quantities and mediation of in vivo cytotoxic effects. With maturing monoclonal antibody technology has come the use of these antibodies in clinical studies in patients with malignancy. These trials have established that monoclonal antibodies can be safely administered in large doses, that their pharmacokinetics and tissue penetration can be predicted and that in some instances a therapeutic effect can be produced by their infusion. A number of problems have also been identified by these studies, including antigenic heterogeneity of the tumor, the presence of free serum antigen, the immunogenicity of the xenogeneic antibody, modulation of the surface antigen by the antibody and a finite capacity of human effector mechanisms to mediate cytotoxicity directed by murine antibodies. Other workers are concurrently investigating the use of monoclonal antibodies in the ex vivo elimination of cells from bone marrow, as probes for serum tumor marker antigens and as carriers for radioimaging agents or toxins. Although most of these endeavors are at the earliest stages, promising preliminary results presage an important role for native and altered monoclonal antibodies in the diagnosis and treatment of malignant conditions.

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In 1906 Paul Ehrlich initially proposed the concept of an antiserum not only specific for a tumor but which after binding to it would mediate its regression.[1] Since that time the search for antiserum fulfilling both these criteria has met with little success in humans. Antiserum obtained from the blood of an immunized animal is heterogeneous, variable from lot to lot and requires extensive absorption with consequent loss of activity to produce specificity. The development of hybridoma technology by Kohler and Milstein in 1975 alleviated these problems.[2] With this technique, lymphocytes from the spleen of a mouse appropriately immunized can be immortalized by fusing them to myeloma cells that have lost the ability to make their own immunoglobulin but are capable of unlimited mitotic division.[3] Through limiting dilution, individual lymphocyte-myeloma hybrids, which produce an antibody of unique specificity, avidity and effector function, can be isolated or cloned from the many hybrids resulting from such a fusion. A cloned hybrid cell line can theoretically produce unlimited quantities of a single antibody of specific and well-characterized binding specificity, avidity and isotype. The capabilities exist to select and produce an antibody fulfilling whatever criteria are deemed important to its purpose. The quantities necessary for therapy in humans or other large-scale application are usually prepared from mouse ascites. These ascites preparations contain varying amounts of mouse albumin, transferrin and nonspecific immunoglobulin after purification. Eliminating contamination by murine viruses, which are potentially pathogenic in humans, is a legitimate concern in ascites-derived antibodies.[4] In vitro methods currently available, however, make the production of gram quantities of monoclonal antibodies of very high purity a practical reality.[5] Because of the monoclonality of the immunoglobulin, no absorption is necessary and virtually all of the antibody in the preparation can be recovered for use. Thus, the production of large quantities of a pharmaceutically pure, highly specific monoclonal antibody is not only a theoretic but a practical reality.

The clinical potential for these reagents in oncology exceeds that of Ehrlich's theoretic antitumor antiserum. As diagnostic reagents, they may help monitor therapy or detect

From the Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford.

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Reprint requests to Ronald Levy, MD, Division of Oncology, Department of Medicine, Stanford University Medical Center, Stanford, CA 94305.

ABBREVIATIONS USED IN TEXT

ADCC = antibody-dependent cellular cytotoxicity
AMIA = antimouse immunoglobulin antibody
CALLA = common acute lymphoblastic leukemia antigen
Ig = immunoglobulin

occult disease. The monoclonal antibodies could be used to detect minute amounts of free tumor-associated antigens in the serum, antigen-positive cells in the blood or tissue biopsy specimen or as in vivo imaging reagents in the form of immunoconjugates. Their specificity could be used to localize conjugated toxic agents to the tumor, thus producing a response. These issues, both realized and potential, are discussed in this article.

Monoclonal Antibody Therapy in Patients With Malignancy

General Considerations of In Vivo Administration

Monoclonal antibodies specific for various malignant conditions are currently being investigated as therapeutic agents in many centers around the United States. Unlike conventional therapy, which can be tested for activity against a variety of malignant tumors, monoclonal antibodies have a predefined potential activity limited to tumors bearing their target antigen on their surface. Whatever the mechanism of an antibody-induced clinical response, we presume that it must result from binding of antibody to the surface of all of the cells in the tumor with proliferative capacity.

The ideal target for a monoclonal antibody would be an antigen expressed in high density on the surface of only tumor cells, that is not secreted or shed as free protein and that does not modulate (see Table 1). This antigen must be on the surface of every tumor cell, especially any stem cell population that exists, and have no capacity for structural change that might alter its reactivity with the antibody. The targets of most antibodies used in therapeutic trials to date are tumor-associated differentiation antigens that are also expressed on certain normal cells, usually in lower density. A search continues for fetal proteins or oncogene expression products on the cell surface of malignant tumors that might be used as discriminating targets.

Tumor Heterogeneity

Subpopulations of the tumor may express a mutated form of the antigen or lack the surface antigen altogether, thus rendering them unreactive with the antibody.[6] Selection of drug-resistant subsets of tumor is a well-known mechanism of escape from chemotherapy. It is only logical that if an effective negative selection force—in this case, monoclonal antibodies—exists, then cells unreactive with the antibody would have a growth advantage and emerge as the predominant population. This phenomenon was noted by Meeker and coworkers in two patients with B-cell lymphoma treated with anti-idiotype antibodies.[7] No unreactive cells were detected before therapy with a single antibody, suggesting that the variants were present at a frequency of less than 1 in 200, the lower limit of detection sensitivity. In this instance, a minor mutation occurred in the expressed surface immunoglobulin, making it unreactive with the anti-idiotype antibody but otherwise similar to the initial idiotypic immunoglobulin. Other

TABLE 1.—*Problems With the In Vivo Use of Monoclonal Antibodies*

Specificity of antibody: tumor-associated versus tumor-specific antigens
Antigenic heterogeneity of the tumor cell population
Extracellular free antigen
Immunogenicity of the mouse immunoglobulin
Modulation
Antibody-host ability to mediate directed cytotoxicity
Nonspecific (Fc-mediated) localization of antibody

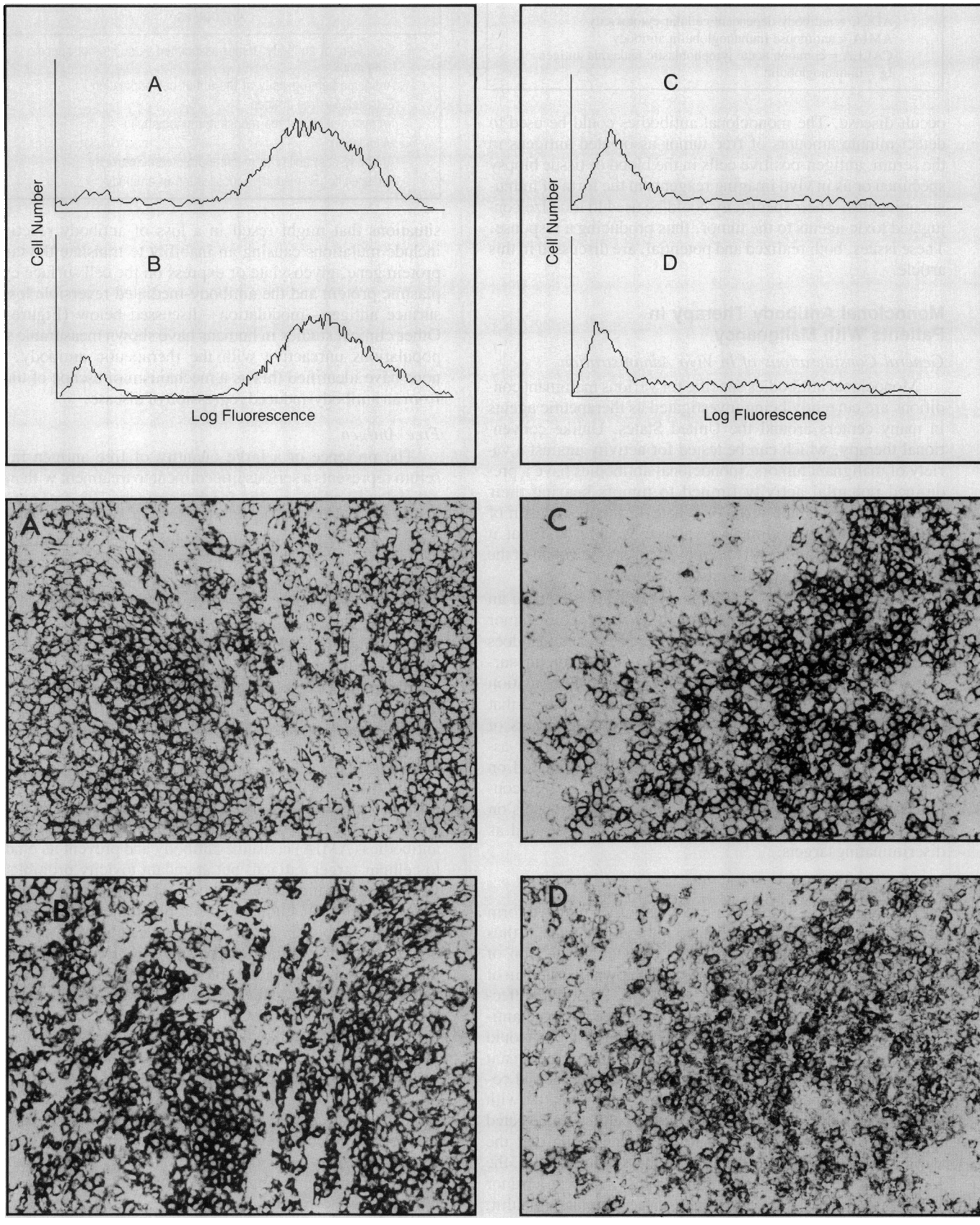
situations that might result in a loss of antibody reactivity include mutations causing an inability to translate the target protein gene, glycosylate or express on the cell surface cytoplasmic protein and the antibody-mediated reversible loss of surface antigen—modulation—discussed below (Figure 1). Other clinical studies in humans have shown measurable subpopulations unreactive with the therapeutic antibody, but none have identified this as a mechanism of escape of tumor from an antibody-induced regression of disease.

Free Antigen

The presence of a large quantity of free antigen in the serum represents a serious impediment to treatment with monoclonal antibodies specific for that antigen. This free antigen would be as likely to bind infused antibody as cell surface antigen and by forming complexes would consume antibody and block penetration and binding of antibody to extravascular tumor. The formation of large amounts of immune complexes might also produce toxicity. Free antigen was noted to be a practical problem in the study by Nadler and associates.[8] The treatment of lymphoma with anti-idiotype was also complicated by the presence of circulating free idiotype protein.[9] Small amounts of free antigen have been detected transiently in other studies but none have proved significant to monoclonal antibody therapy.[10,11]

Immunogenicity

Control of the host response against the xenogeneic antibody is important to the ultimate success of monoclonal antibody therapy. Not only do the antimouse immunoglobulin antibodies (AMIA) consume antibody and prevent its binding to cellular target antigen, but attendant toxicity prohibits infusing large quantities of antibody.[9] The incidence of AMIA varied in different studies, depending on the immunocompetence of the subjects. AMIA is first detectable between 7 and 30 days after the initial dose of antibody. It reacts with both the constant region of the antibody and its variable region (idiotype).[9,12-14] As patients with less advanced disease are treated, their immune systems might be expected to be more competent and the likelihood of AMIA developing may be greater. The use of concurrent therapies to suppress the response or produce immunologic tolerance has shown some effects but is not yet practical.[12] The initial administration of large doses of highly purified monoclonal antibodies may induce "high zone tolerance," a suppression of the antibody response by very large doses of antigen.[15] The development of less immunogenic human monoclonal antibodies or recombinant chimeric antibodies might minimize the importance of this problem, if anti-idiotype antibodies are not produced.[16,17]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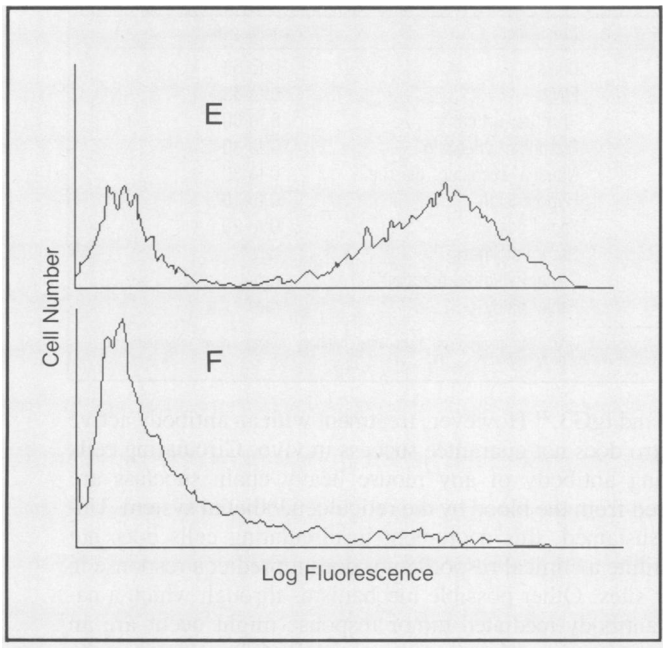

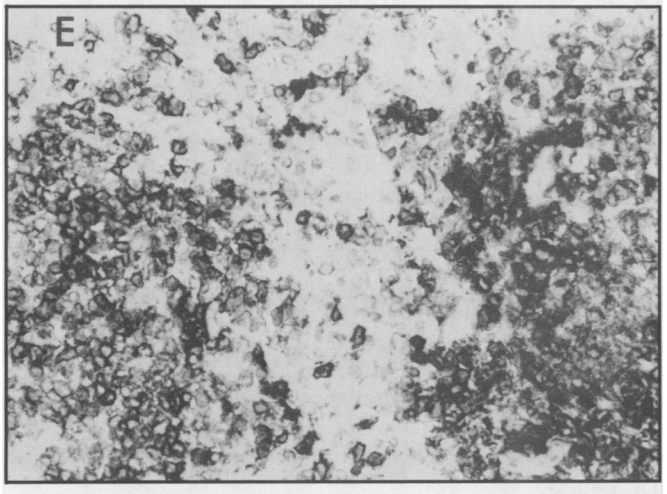

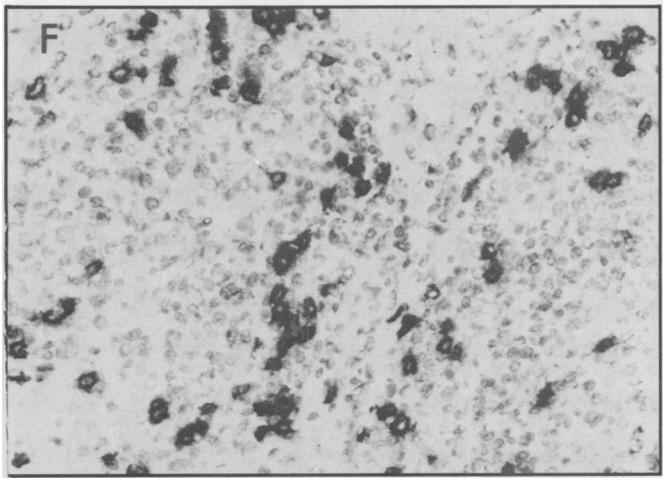

Figure 1.—Comparison of flow cytometry fluorescence histograms of single-cell suspensions with immunoperoxidase staining of frozen tissue sections. These represent the two most commonly used methods of assessing antibody reactivity with cellular antigens. **A** (anti-μ) and **B** (anti-idiotype) represent staining with two antibodies specific for different epitopes of the surface immunoglobulin of a B-cell lymphoma before treatment with anti-idiotype antibody. A gaussian distribution of each cell population is seen in the fluorescence histograms, the brightest staining—or "positive"—cells on the right of each curve, the dullest staining—"negative"—on the left. The frozen sections are stained with the appropriate mouse antibody and then detected with an antimouse immunoglobulin (Ig) avidin-biotin peroxidase method. The dark precipitate identifies cells that bind the antibody of interest. The staining patterns using both methods are the same for both antibodies before therapy. **C** (anti-μ) and **D** (anti-idiotype) represent staining 18 hours after a large dose of anti-idiotype antibody. The fluorescence histograms show a greatly decreased number of positive cells for both antibodies. This is caused by *modulation,* antibody-mediated internalization of the surface immunoglobulin. The tissue sections show continued reactivity with both anti-μ and anti-idiotype antibody, perhaps because this method detects both surface and cytoplasmic immunoglobulin. Thus, modulation is better assessed with flow cytometry than with tissue sections. Discordance of reactivity with the two antibodies is seen in a posttherapy tumor specimen (panels **E** [anti-μ] and **F** [anti-idiotype]). Although both surface and cytoplasmic IgM are detectable, only a minor population of tumor cells reacts with the anti-idiotype antibody. This is consistent with heterogeneity of the tumor cells that developed after selection of an antigen-negative subpopulation by the anti-idiotype therapy. (All photomicrographs are original magnification × 350, courtesy of Carlos Garcia and Roger Warnke.)

Modulation

Modulation is the term that describes the coalescence of surface antigen cross-linked by multivalent antibody followed by internalization of the complex.[18] This renders the modulated cell surface antigen-negative and, thus, resistant to serotherapy by antibodies against that antigen.[19] On the other hand, if the antibody is conjugated to a toxin, internalization of the antigen-antibody complex may potentiate the effects of the "armed" antibody. Modulation of one surface antigen does not appear to affect other cell surface proteins and the malignant cells can still be identified using other specific surface antigens. Once antibody is not present, the modulated antigen will be reexpressed. In general, antigens on the surface of lymphoid cells are more easily modulated than those on solid tumors.

Toxicity

Intravenous monoclonal antibody infusions are frequently associated with toxicity that is tolerable in most cases and with careful attention can be minimized. Virtually all studies agree that the amount of toxicity depends on the amount of intravascular target antigen, whether it is in the form of free antigen, antigen-bearing cells or AMIA.[9,20,21] This suggests that immune complexes or cellular immunoaggregates produce the toxic effects, although this has not been proved. Idiosyncratic anaphylactoid reactions are rare and have never been described in the absence of an intravascular target. Local reactions to intradermal injection or a scratch test with the monoclonal antibody failed to predict systemic reactions. Pretreatment with acetaminophen and diphenhydramine hydrochloride mitigates the symptoms associated with reactions. The most common symptoms include chills, fever, hyperemia of the conjunctivae and mucous membranes, urticaria, periorbital edema, dyspnea, wheezing, hypotension, nausea and emesis (Table 2). Interrupting the antibody infusion is the most useful measure to control these symptoms when they occur. Parenteral administration of diphenhydramine, meperidine hydrochloride (for rigors) and prochlorperazine is useful to alleviate specific problems. In some studies 24-hour infusions were used to limit toxicity that appears to be infusion-rate dependent. These symptoms seem to occur at an equivalence point, and when the infused antibody is in excess in the serum, the infusion may proceed without side effects. Toxic reactions seldom prevent the infusion of even large doses of monoclonal antibody. The most severe reported toxicity was associated with infusion of large doses of antibody in the presence of established AMIA.[9]

Host Immune System and Mechanism of Response

The finite capacity of the host-effector mechanisms that are directed by the antibody to produce cytotoxicity may ultimately be the major limitation to therapy for malignant tumors with native murine monoclonal antibodies. The proposed mechanism of antitumor effect in human trials is based on results in studies using animals, primarily mice.[22] The destruction of an antibody-coated cell in vitro is mediated either by complement (cell-mediated cytotoxicity) or Fc receptor-bearing cells (antibody-dependent cellular cytotoxicity [ADCC]). Studies of ADCC with murine antibodies and human effector cells suggest that immunoglobulin (Ig)[23,24] G2a and IgG3 are the most effective murine heavy chain isotypes. Human complement fixation is best achieved by IgM and IgG3.[25] However, treatment with an antibody active in vitro does not guarantee success in vivo. Circulating cells binding antibody of any mouse heavy chain subclass are cleared from the blood by the reticuloendothelial system. Unless sustained, this decrement in circulating cells does not constitute a clinical response nor does it predict a response in other sites. Other possible mechanisms through which a native antibody-mediated tumor response might occur are an antiproliferative effect (anti-Ig, anti-DR, G5) or blocking the binding of a substance necessary to support the growth of the cell (antiepidermal growth factor, antitransferrin receptor, anti-B cell growth factor).[26-31]

TABLE 2.—*Toxicity of Monoclonal Antibody Infusions*

Toxic Effect	*Frequency (%)*
Chills	0 to 63
Fever	0 to 63
Pruritus, urticaria	5 to 80
Dyspnea, wheezing	0 to 30
Nausea, vomiting, diarrhea	0 to 30
Hypotension	0 to 10
Facial edema	0 to 20
Serum sickness	rare*
"Anaphylactoid reaction"	rare
Facial palsy	rare

*Observed only after infusion of monoclonal antibody during active antimouse immunoglobulin antibody response.

Clinical Trials

Malignant Hematolymphoid Disorders

Most of the clinical trials with monoclonal antitumor antibodies have been done in patients with leukemia or lymphoma. The accessibility of tumor cells in the blood allows convenient assessment of both antibody binding and response to therapy. Large numbers of antibodies have been developed against normal and malignant lymphoid cells. The monoclonal antibodies used in these trials have represented both extremes in specificity, from differentiation antigens present on normal cells, such as the T65 antigen, to antibodies specific for a unique tumor in a single patient, or anti-idiotype antibodies.[9,10,20,21,32,33] The results of these trials are summarized in Table 3.

T101 and anti-Leu-1 antibodies are specific for the T65 antigen present on both malignant and normal T cells and a subset of normal and malignant B cells. These antibodies were used to treat patients who have cutaneous T-cell lymphoma and chronic lymphocytic leukemia, a B-cell malignant disorder in which the T65 antigen is expressed.[10,20,21,32,33] These patients were refractory to conventional therapy. Infusion of either antibody led to the elimination of circulating antigen-positive cells from the blood. This elimination of cells was usually transient, the tumor cells reappearing in the blood within 24 hours. Doses of antibody, all delivered intravenously, varied from 1 to 600 mg. With increasing doses of antibody, saturation of the antigen on circulating cells was achieved and free antibody was ultimately detectable in the serum. Once the cells in the blood were saturated and antibody excess was achieved, modulation of surface antigen occurred and antigen-negative tumor cells could be identified in the blood. When free antibody was no longer detectable in the serum, surface antigen-bearing cells were again present in

TABLE 3.—*Clinical Trials With Monoclonal Antibodies Specific for Hematolymphoid Malignancy*

Institution	Disease	Monoclonal Antibody	Dose (mg)	AMIA*	Tumor Response†	Sources
Dana-Farber‡	Lymphoma	AB89	25 to 150	0	0	Nadler et al, 1980[8]
Dana-Farber	ALL	J5	1 to 175	ND	0	Ritz et al, 1981[19]
UC San Diego§	CLL	T101	10 to 500	0/6	0/6	Dillman et al, 1984[32]
NCI‖	CLL	T101	1 to 100	0/13	0/13	Foon et al, 1984[20]
Stanford¶	CTCL	Leu 1	1 to 600	6/13	8/13	Miller et al, 1983[10]
UC San Diego	CTCL	T101	10 to 500	3/10	5/10	Dillman et al, 1984[32]
NCI	CTCL	T101	1 to 500	4/7	ND	Foon et al, 1983[33]
Stanford	Lymphoma	Anti-idiotype	1 to 900	5/10	6/10	Meeker et al, 1985[9]

ALL = acute lymphoblastic leukemia, AMIA = antimouse immunoglobulin antibody, CLL = chronic lymphocytic leukemia, CTCL = cutaneous T-cell leukemia/lymphoma, ND = no data

*Incidence/evaluable patients in the study.
†Number of patients with objective tumor response/ evaluable patients in the study.
‡Dana-Farber Cancer Institute, Boston, Mass.
§University of California, San Diego, Medical Center, San Diego, Calif.
‖National Cancer Institute, Washington, DC.
¶Stanford University Medical Center, Stanford, Calif.

the peripheral blood. Low-dose intermittent therapy was thus believed to maximize the antitumor effect while minimizing modulation. Penetration of extravascular tumor sites was documented by both Miller and associates and Foon and colleagues, usually by the occurrence of modulation rather than detectable mouse immunoglobulin.[10,20]

Although most of the toxic effects listed in Table 2 were observed, dyspnea was the most common and believed to be due to agglutination of antigen-positive cells. This was associated with a transient lung scan abnormality in one instance.[20] In more than half of the patients with cutaneous T-cell lymphoma, antimouse immunoglobulin antibodies ultimately developed, in contrast to none of the patients with chronic lymphocytic leukemia. When anti-T-cell antibodies were infused into normal primates, all recipients made AMIA.[12,34] This suggests that in the patients studied, the underlying disease and its prior therapy resulted in immunosuppression that reduced the incidence of AMIA. Clinical responses were limited to transient clearance of cells in patients with chronic lymphocytic leukemia. Half the patients with cutaneous T-cell lymphoma had objective responses including reductions in lymphadenopathy, circulating cell numbers and skin disease (Table 3). These responses were of 1.5 to 4 months' duration and often interrupted by increasing amounts of AMIA.

Ritz and co-workers reported on the treatment of four cases of relapsed common acute lymphoblastic leukemia antigen (CALLA)-positive acute lymphoblastic leukemia with J5, an IgG2a murine monoclonal antibody specific for CALLA.[19] Although dramatic reductions in circulating lymphoblasts could be observed during antibody infusions, the return of the lymphoblasts was quite rapid, and no decrease in the bone marrow infiltration was noted. Like the T65 antigen, CALLA rapidly disappeared from the surface of the malignant cells in the presence of anti-CALLA and this modulation was believed to interfere with the antitumor effect.

Antibodies against most differentiation antigens, like those discussed, presumably produce their antitumor effects by mediating cell-mediated or antibody-dependent cellular cytotoxicity (ADCC). As mouse antibodies (except perhaps IgG3) are poor at fixing human complement and only selected IgG2a and IgG3 antibodies are efficient at producing in vitro ADCC, the lack of major clinical efficacy in the studies is perhaps not surprising. Currently, trials with the Leu-1 and T101 antibodies have been discontinued. Trials with T101 antibody conjugated to drugs or toxins are contemplated.

At Stanford, 13 patients (11 previously reported) with B-lymphoid malignant disorders have been treated with monoclonal antibodies specific for the variable region or idiotype of the clonal immunoglobulin expressed on the surface of these tumors.[9] Anti-idiotype antibodies must be "tailor-made" for each patient's tumor. In these trials, IgG1, IgG2b and IgG2a antibodies have produced tumor responses in some patients. Significant amounts of paraprotein, as well as idiotype-bearing cells, may interfere with anti-idiotype therapy. Free serum idiotype antigen must be decreased either by plasmapheresis or by reducing tumor bulk. Single intravenous doses of 1 to 1,500 mg have been administered. The cumulative dose has varied from 400 to 9,250 mg of immunoglobulin. Toxic reactions were similar to those seen with anti-Leu-1 antibody and characterized by chills, fever, transient thrombocytopenia and occasional rash. The two patients who received large doses of antibody while an AMIA developed experienced the most severe toxic reactions. One of these patients had profound neutropenia during the infusions and had diffuse rashes and arthralgias. Another of these patients became hypotensive and acute tubular necrosis and a seventh cranial nerve palsy developed. In later patients the initial doses of antibody have been increased to 400 to 600 mg and no AMIA has been detected. Seven of twelve evaluable patients have had objective tumor regressions lasting from one to six months. In two of these patients tumor populations emerged that did not react with the anti-idiotype antibody.[7]

Melanoma

Melanoma has long been the subject of immunotherapeutic investigations. In malignant melanoma, there is no effective conventional therapy for metastatic disease. In rare cases, however, there are spontaneous regressions, suggesting some type of host control. Tumor nodules on the skin are accessible for biopsy and evaluation of response. A large number of surface antigens has been defined and a variety of monoclonal antibodies has been developed against each. Several centers have recently reported clinical trials with such antimelanoma antibodies (Table 4).[25,35,36] These trials have been important because they have established a number of

TABLE 4.—*Clinical Trials With Antibodies Against Solid Tumor Antigens*

Institution	Tumor Diagnosis	Monoclonal Antibody	Isotype	ADCC	Complement		Dose, mg	AMIA*	Tumor Response†	Source
					Human	Rabbit				
Sloan-Kettering‡	Mel	R_{24}	IgG3	3+	4+	...	8 to 240	+	5/12	Houghton et al, 1985[25]
NCI§	Mel	9.2.27	IgG2a	...	...	...	1 to 200	+	0/8	Oldham et al, 1984[35]
Washington‖	Mel	96.5	IgG2a	+/−	0	3+	1 to 424	...	0/5	Goodman et al, 1985[36]
	Mel	48.7	IgG1	0	0	0	1 to 212	...	0/4	Goodman et al, 1985[36]
Wistar¶	GI	17-1A	IgG2a	2+	...	...	10 to 1,000	+	3/20	Sears et al, 1982[37]
UCLA**	GI	CCOLI	IgG3	3+	4+	...	5 to 3,000	+	0/8	Lemkin et al, 1984[39]

ADCC = antibody-dependent cell-mediated cytotoxicity, AMIA = antimouse immunoglobulin antibody, GI = gastrointestinal cancer, Mel = malignant melanoma

*+ = AMIA detected in all patients.
†Objective tumor response/evaluable patients.
‡Memorial Sloan-Kettering Cancer Center, New York, NY.
§National Cancer Institute, Washington, DC.
‖University of Washington/Harborview Medical Center, Seattle.
¶The Wistar Institute of Anatomy and Biology, Philadelphia.
**UCLA Jonsson Comprehensive Cancer Center, Los Angeles.

facts about therapeutic monoclonal antibodies concerning the safety of administration, tissue penetration, pharmacokinetics, immunogenicity and efficacy. No intravascular antigen was detectable in any of these studies, allowing accurate determination of the dose level necessary to penetrate extravascular solid tumor sites. Single doses of more than 50 mg routinely penetrate to skin tumor nodules, initially binding to cells in the perivascular areas 16 hours after a dose. Single doses of 200 mg did not saturate the tumor cells, but ten 25-mg doses a day ultimately resulted in saturation of the tumor cell surface antigen. No modulation of these antigens was observed. The half-life of these antibodies varied between 21 and 53 hours. All of the different antibodies evoked AMIA responses. AMIA occurred transiently in one of eight patients in the National Cancer Institute study and virtually all of the patients in the other studies. These AMIA blocked in vitro binding of antibody to antigen. Most important are the differences in efficacy observed. These differences correlate with certain in vitro studies done of the therapeutic antibodies. Only R_{24}, an IgG3 antibody that mediates ADCC with human effector cells and fixes human complement in vitro, produced clinical responses. The other three antibodies of IgG1 and IgG2a subclasses were ineffective both in vitro and in vivo. These antibodies recognized different cell surface antigens. The effective antibody binds to Gb3, a ganglioside, in contrast to the glycoproteins recognized by the other antibodies that have been tested clinically.

Gastrointestinal Malignancy

In 1982, Sears and colleagues described patients treated with 17-1A, an IgG2a monoclonal antibody specific for an antigen expressed on the surface of malignant gastrointestinal tumors.[37] More than 20 patients with colonic, rectal, gastric or pancreatic tumors have received single doses of 17-1A varying from 15 to 1,000 mg.[15] Three patients with hepatic metastasis were infused via the hepatic artery, the remainder received antibody intravenously over 30 to 60 minutes. Toxic reactions were minimal, with one patient experiencing wheezing and a vasoactive reaction when infused in the presence of an AMIA response resulting from a previous administration of antibody for imaging. An interesting observation was a significant decrease in the incidence of AMIA in patients receiving large doses of antibody. In only one of nine infused with doses greater than 200 mg did AMIA develop, compared with eight of nine patients receiving a smaller dose. This might represent high zone tolerance in these patients and may signal an important approach to limiting AMIA in patients treated with other antibodies.[15] This antibody can mediate ADCC using human effector cells.[38] Because of the nature of these tumors, primarily the lack of accessible evaluable tumor and the often slow growth rate, it is difficult to evaluate the impact of antibody on the tumor in the published trials.

Another antibody that acts against a gastrointestinal tumor-related antigen is CCOL1.[39] This antibody is an IgG3 murine antibody that fixes human complement and has antibody-dependent cell-mediated cytotoxicity. Doses of up to 3 grams were infused in eight patients without severe toxic reactions. Profound declines in the C3, C4 and CH_{50} unit levels were observed during infusion of this antibody. The lack of clinical responses was perhaps related to inadequate amounts of complement, although that was not the case with R_{24}, another IgG3 antibody (antimelanoma).

Bone Marrow Transplantation: Selective Elimination of Cells In Vitro

Bone marrow transplantation has proved to be a useful treatment modality for leukemia, lymphoma and some solid tumors.[40,41] A major impediment to the use of these transplants has been the presence of contaminating tumor cells in autologous marrow or donor lymphocytes that produce graft-versus-host disease in allogeneic marrow. Monoclonal antibodies are very effective in selectively eliminating target cells from single cell suspensions in vitro. This property has been exploited to process harvested bone marrow to remove contaminating tumor cells from autologous bone marrow or T lymphocytes from allogeneic bone marrow.[42–44]

Processing of marrow must occur before cryostorage as viability will be compromised by postthaw manipulation. Antibodies plus rabbit complement or antibodies bound to a solid phase such as Sepharose (an agarose gel) or magnetic beads will remove up to three logs of unwanted cells without decreasing the viability of the remaining cells. Several sequential treatments are more effective than one. By using several antibodies specific for different antigenic determinants, more complete elimination can be achieved than with a single antibody.[42] Antibody conjugated to some toxic moiety may eliminate cells through cytotoxicity.[45]

Several institutions are conducting trials using purged bone marrow.[46-50] Hematologic recovery has not been a problem in those patients receiving treated autologous marrow. In some cases in which allogeneic marrow has been treated to minimize graft-versus-host disease, the donor marrow has failed to engraft.[50] As this problem is further studied, antibodies or procedures (or both) that selectively eliminate cells producing graft-versus-host disease and retain cells necessary for engraftment will be identified and used for purging allogeneic marrow. Unfortunately, the efficacy of autologous marrow-purging techniques is difficult to evaluate, as diseases treated in this manner are often refractory to the preparative regimen and the patient can relapse from residual disease as well as from the reinfused marrow. In addition, no data exist regarding the minimal number of contaminating malignant cells that will reseed a patient with tumor.[43]

Diagnostic Use of Monoclonal Antibodies

Imaging

Although still in the earliest phases of development, the use of specific monoclonal antibody-radionuclide conjugates is very promising. The staging information that could be obtained would influence the management of diseases such as breast cancer or melanoma through accurate noninvasive staging at diagnosis. Antibodies such as 17-1A (specific for gastrointestinal cancer), 9.2.27 and 96.5 (antimelanoma), 3F8 (antineuroblastoma) and T101 have been used to image their respective tumors after intravenous administration.[35,51-54] The problems that must be overcome to make these antibodies clinically useful are elimination of Fc binding and other nonspecific adherence, immunogenicity and ease of radionuclide conjugation. Solving these problems would eliminate background and false-positives and allow repeated administrations over time. A large variety of approaches to these problems exists and the most practical and effective must be chosen from among them.

A novel use of radionuclide-labeled antibodies is local lymphatic imaging.[55] After subcutaneous injection, monoclonal antibodies directed against a tumor entered local lymphatic vessels, passed to the draining lymph nodes and bound to localized metastatic lesions. This approach minimizes many of the problems encountered with intravenous injection, and local metastases are imaged, which is useful for staging in diseases such as breast carcinoma and melanoma. If adequate sensitivity and specificity of the data generated by this approach are achieved, surgical staging may become unnecessary.

Serum Tumor Markers

The serum concentration of tumor markers such as β-human chorionic gonadotropin and α-fetoprotein for testicular cancer and paraprotein for plasma cell myeloma reflects tumor bulk and the presence of occult disease. Bast and associates reported that blood levels of an ovarian carcinoma-associated-antigen correlated with the relative mass of ovarian tumor.[56] The small amounts of paraprotein secreted by most patients with B-cell lymphoma were undetectable using the usual clinical immunologic methods but easily quantitated with specific anti-idiotype antibody.[9] The serum concentrations of these paraproteins reflected tumor bulk.[9] As enzyme-linked immunosorbent assays are sensitive to 10 ng per ml, only small amounts of antigen need be present if the detecting antibody is specific. As a measure of occult disease, these tumor markers could be valuable in the management of certain malignant disorders.

The Future of Monoclonal Antibodies

At this time only modest advances in the care of patients with cancer have occurred as a result of monoclonal antibodies. However, in a very brief period a great deal has been learned about these proteins despite their limited use in patients. Technology has enabled us to produce the desired specificity in these antibodies but not necessarily the activity. Most of the clinical and laboratory study of antibodies in the near future will be devoted to modifications of the Fc portion of the immunoglobulin molecule designed to reduce nonspecific binding or immunogenicity or to arm the antibody with toxins, radionuclide reagents or a more effective Fc.

The most common modification of the Fc for therapeutic purposes has been to conjugate extremely potent toxins to the antibodies. One molecule of biologic toxins such as ricin, abrin or diphtheria toxin can theoretically result in the demise of a cell.[57,58] These toxins are composed of a cytotoxic A chain and a B chain that nonspecifically bind to galactose residues on the cell surface. Problems related to the nonspecific binding of the intact toxin molecule and the loss of the A-chain cytotoxic activity when the binding (B) chain is eliminated must be solved before immunotoxins are practical for in vivo administration. Ex vivo processing of bone marrow with immunoconjugates can be safely accomplished using various maneuvers not practical in vivo to protect the desired subpopulations of cells while destroying the cells recognized by the antibody.[57] Conversely, with appropriate antibodies, pluripotent stem cells desired for reinfusion into patients could be positively selected from the bone marrow. Antibodies labelled with high-specific-activity isotopes might effectively treat tumors in which the antibody localizes.[59] Isotopes that upon decay emit α or β particles would be cytotoxic to within several cells of the site to which it binds, thus limiting toxicity to adjacent normal tissues. Nonspecific accumulation of the antibody-isotope conjugate in the liver and spleen with attendant toxicity will likely result with the immunoconjugates currently available.

Other modifications of the Fc portion that have yet to be used in clinical trials are designed to reduce the immunogenicity and nonspecific binding or increase the efficacy of the antibody. Antibodies with polyethylene glycol polymers conjugated to the constant region were nonimmunogenic and exhibited minimal nonspecific binding.[60] Chimeric antibodies, produced using recombinant DNA techniques, combine the variable region of a mouse hybrid with a human Fc.[16,17] The human Fc should be nonimmunogenic and will likely mediate human effector activity much more effectively than mouse Fc. Human-human hybridomas secreting entirely human antibody could, in theory, provide the optimal monoclonal antibodies for use in humans, but several problems still exist. A practical fusion partner is not yet available. More important, no method exists for producing immunized human lymphocytes secreting antibody of a desired specificity.

Many tests will be done in large in vitro screenings to identify antibodies that will fulfill defined purposes. Because

the toxicity of antibody infusion is low and predictable and specificity predefined, future clinical trials of monoclonal antibodies must necessarily focus on efficacy. Native antibodies of interesting specificity that have no easily demonstrable in vivo antitumor activity should undergo Fc modifications, as described above, early in their development. As technology turns theory into reality, the number of useful therapeutic and diagnostic monoclonal antibodies will rapidly expand. These new cancer-specific agents can be applied in conjunction with conventional approaches for the benefit of an increasing number of patients with cancer.

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